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BRNO UNIVERSITY OF TECHNOLOGY

FAKULTA CHEMICKÁ
ÚSTAV FYZIKÁLNÍ A SPOTŘEBNÍ CHEMIE

FACULTY OF CHEMISTRY
INSTITUTE OF PHYSICAL AND APPLIED CHEMISTRY

DYNAMIC TENSIOMETRY OF BIOCOLLOIDS

DIPLOMOVÁ PRÁCE
DIPLOMA THESIS

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AUTHOR

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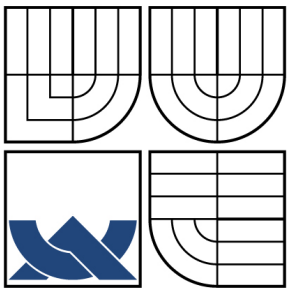

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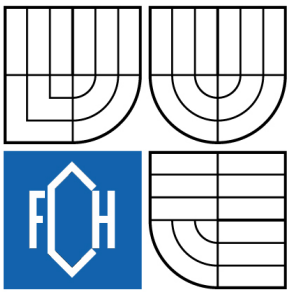

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ABSTRACT

Hyaluronic acid is currently one of the biomolecules with great interest which is widely used in medicine and cosmetics and its investigation is very important for future use. The aim of this work was to investigate the surface behavior, using dynamic tensiometry method, of different systems namely hyaluronic acid, its hydrophobic derivatives and SDS solutions for comparison. These compounds were investigated in water and in sodium counterions. The observed systems were measured in various concentration ranges under the laboratory temperature. It was performed two methods employing a BPA-800P Bubble Pressure Tensiometer which is completely new apparatus and new technique of tensiometry measuring. There were proposed some experiments for biocolloids research to find usable possibilities of this apparatus for next research works. Results of thesis show single surfactants differences depending on their concentration and used environment. Hyaluronic acid exhibits no surface activity in contrast to its derivatives and SDS.

ABSTRAKT

Hyaluronová kyselina je v současné době velmi významná biomolekula používaná v medicíně a kosmetice, a její výzkum je důležitý pro další budoucí použití. Zaměření této práce je studování povrchových vlastností hyaluronové kyseliny, jejích hydrofobních derivátů a roztoků SDS pro srovnání, za použití tenziometrie. Tyto sloučeniny byly zkoumány ve vodě a v roztocích ve formě sodné soli. Sledované vzorky byly měřeny dvěmi metodami v různých koncentračních rozmezích při laboratorní teplotě pomocí nového BPA-800P bublinového tenziometeru. Byly navrženy takové experimenty, aby se zjistily využitelné možnosti tohoto přístroje pro další výzkum. Výsledky práce ukazují rozdílnosti jednotlivých povrchově aktivních látek v závislosti na jejich koncentraci a použitém prostředí. Hyaluronová kyselina nevykazuje povrchovou aktivitu, zatímco její deriváty a SDS ano.

KEYWORDS

hyaluronic acid, dynamic tensiometry, biocolloids, maximum bubble pressure method

KLÍČOVÁ SLOVA

hyaluronová kyselina, damická tenziometrie, biokoloidy, metoda maximálního tlaku v bublině

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DECLARATION

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PROHLÁŠENÍ

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podpis studenta

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1 INTRODUCTION

This thesis deals with the dynamic tensiometry, especially surface tension of hyaluronic acid and its derivatives. Hyaluronic acid is natural polysaccharide important for the living tissues in every vertebral body. It makes hydration, easy joint move, injury health and cell rigidity. It's very important subject in modern medicine. Dynamic tensiometry includes many methods for measuring surface tension by a tensiometer. In this case is the maximum bubble pressure tensiometer used. Hyaluronic acid and its derivatives surface tension depends mainly on their concentration and environment of the solution. Differences between hyaluronic acid and derivatives surface tension are studied. With increasing concentration decrease the derivatives surface tension. It depends also on the surfactants molecular mass. SDS surface tension was measured too. This well known and examined surfactant allowed surface tension comparison with hyaluronic acid and its derivatives.

2 STATE OF THE ART

Mobile interface of phases exists between the liquid and gas phases or liquid and liquid phases. These interfaces are homogeneous and the interfacial energy is good measured. For phases between the gas and liquid we use name *surface free energy*, or *surface tension* [1]. It is an effect within the surface layer of a liquid that causes that layer to behave as an elastic sheet. It is a measurement of the cohesive energy present at an interface, which is caused by the attraction between the molecules of the liquid by various intermolecular forces [2].

In the bulk of the liquid each molecule is pulled in all directions by neighbouring molecules. At the surface of the liquid, the molecules are pulled inwards by other molecules deeper inside the liquid and are not attracted as intensely by the molecules in the neighbouring medium. So all the molecules at the surface are subject to an inward force of molecular attraction which can be balanced only by the resistance of the liquid to compression. This inward pull tends to minimalization the surface area. Thus the liquid squeezes the surface together until it has the locally lowest surface area possible [2][4].

The net effect of the molecules is the presence of free energy in the system. Intermolecular interactions leads to the change of surface free energy. The surface tension can be quantified as a measurement of energy/area. The common units for surface tension are J/m^2. The more dense fluid is heavy phase and the less dense fluid is the light phase [5].

Polar liquids, such as water, have strong intermolecular interactions and thus high surface tensions. Any factor which decreases the strength of this interaction will lower surface tension. Thus an increase in the temperature of this system will lower surface tension. Any contamination, especially by surfactants, will also lower surface tension [5].

Equation for surface tension of liquids – *Eötvös equation* is most known in form:

$$\gamma = \left(\frac{M}{\rho_l} \right)^{\frac{2}{3}} = k \cdot \left(T_c - T \right), \tag{1}$$

here γ is the surface tension measured in N/m. Especially for liquids mN/m because of its low value. M is the molar weight, ρ_l is liquid density, k is empiric parameter and T_c is critical temperature [6].

This surface tension value is numerically and dimensional equal to *interfacial energy*. It is work needed to reversible and isothermal formation of phase interface unit area. Molecules from inwards go to the interface of phases [7].

$$\gamma = \left(\frac{\partial F}{\partial A} \right)_{T,V,n_i} = \left(\frac{\partial G}{\partial A} \right)_{T,p,n_i}, \tag{2}$$

where ∂F and ∂G is the Helmholtz and Gibbs energy change [6].

Gibbs adsorption isotherm is also important because of the relative adsorption $\Gamma_{2,1}$, which presents element 2 adsorption value.

$$\Gamma_{2,1} = -\frac{1}{RT}\left(\frac{\partial\gamma}{\partial \ln a_2}\right)_{T,p} = -\frac{a_2}{RT}\left(\frac{\partial\gamma}{\partial a_2}\right)_{T,p}, \quad (3)$$

where a_2 is activity of element 2 [6].

2.1 Tensiometry

There has been some measuring methods. At first the *static methods*, such as capillary elevation analysis, pendant drop shape analysis or Wilhelmy balancing plate analysis. Second are the *dynamic methods*, this is for example the oscillating beam analysis. Finally third are the *semistatic methods*. Here exists the drop weighing analysis, du Noüy ring analysis, and the maximum bubble pressure analysis [1].

2.1.1 The maximum bubble pressure method [MBMP]

An method for determining the dynamic surface tension is the method of measuring the maximum bubble pressure. In a bubble pressure tensiometer gas bubbles are produced in the sample liquid at an exactly defined bubble generation rate. The gas bubbles enter the liquid through a capillary whose radius is known. During this process the pressure passes through a maximum whose value is recorded by the instrument [8].

The principle of the BPA-800P is shown in the schematic here (Figure 1) [9]:

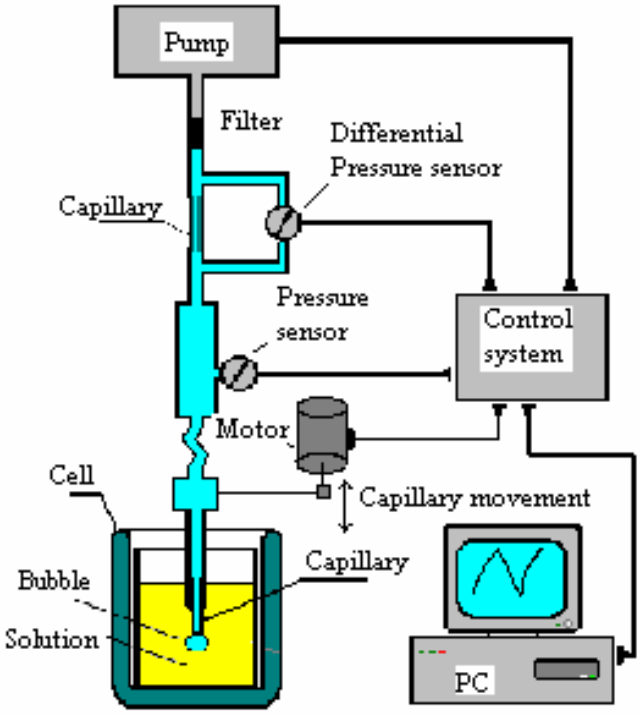

Figure 1 *Schematic representation of device*

The pump produces a continual gas flow, which is measured by the gas flow sensor. The gas flow capillary together with the gas volume damps the system and allows a smooth and regular bubble formation. The pressure sensor measures the pressure in the gas volume, which is proportional to the maximum pressure at the capillary tip. The pump and the two sensors are controlled by a computer via an electronic interface board. The PC also collect the measured data, calculates the dynamic surface tension and effective time and presents all results online. Only data in terms of the effective time can be used for compare and for complementation with data from other instruments [9].

At first the bubble is formed. Initially the pressure is below the maximum pressure, the radius of curvature of the air bubble is larger than the radius of the capillary. Than the pressure curve passes through a maximum. At this point the air bubble radius is the same as that of the capillary. After the maximum the dead time of the measurement starts. The pressure decreases again, the radius of the air bubble becomes larger. Finally the bubble escapes from the capillary and rises. The cycle begins again with the formation of the next bubble (Figure 2) [8][9].

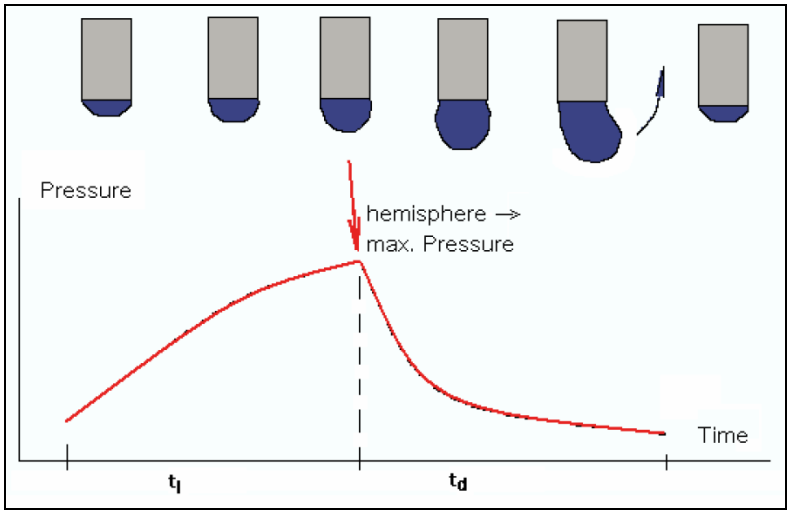

Figure 2 *Schematic of the bubble formation process and the change in capillary pressure*

The t_l is bubble life time and the t_d is dead time. The sum of both times is the bubble time t_b.

Used apparatus for surface tension measuring is the *BPA-800P bubble pressure tensiometer*. There are four methods for measuring:

- standard experiment,
- experiment with given constant life time,
- experiment with increasing gas flow rate value,
- accelerated experiment – Fast scan.

Standard experiment takes 20 – 30 minutes. Starts on t_l = 10ms, and than decelerate the flow rate in steps. Intervals between the bubbles increase. This experiment is mostly used for measuring our surfactants.

Experiment with given constant life time is continual, and is efficient for control processes. User only adjust the t_l.

Experiment with increasing gas flow rate value is right for foamy solutions with increasing flow rate. Maximum of t_l is 5 seconds.

Accelerated experiment – Fast scan is identical to standard experiment, is 3 – 5 times faster. It takes 6 – 8 minutes [9].

For γ calculating is used the Laplace equation by the apparatus.

$$\gamma = f\frac{rP}{2}\ [\text{mN/m}], \tag{4}$$

where f is the correction factor calculated by Sugden, needed in our case when capillary with radius $r > 0.1$mm is used [10].

2.2 Surfactants

Surfactants, also known as tensides, are wetting agents that lower the surface tension of a liquid. This are usually organic amphiphilic compounds. It means they contain both hydrophobic groups and hydrophilic groups. Therefore, they are soluble in both organic solvents and water [11].

Surfactant solutions require a much longer time than water and other liquids to achieve dynamic equilibrium. This is because of their molecular construction. Interfaces are produced extremely quickly in processes such as foaming, cleaning, printing or coating. In such processes it is not just the equilibrium value of the interfacial tension that is the decisive influence, but also the kinetics of the interface formation [8].

The molecular mobility of the surfactants used assumes a considerable influencing factor on the formation of the surface tension. The equilibrium value of the surface tension decreases as the number of surfactant molecules accumulating at the surface increases. It achieves its final value when the surface is completely occupied and offers no place for further molecules [8].

If the concentration is further increased from this point then the surfactant molecules will accumulate within the solution and form aggregates, the so-called *micelles* (Figure 3) [12]. An amphiphilic molecule can arrange itself at the surface of the water such that the polar part interacts with the water and the non-polar part is held above the surface. The presence of these molecules on the surface disrupts the cohesive energy at the surface and thus lowers the surface tension. Molecules can form aggregates in which the hydrophobic portions are oriented within the cluster and the hydrophilic portions are exposed to the solvent [12].

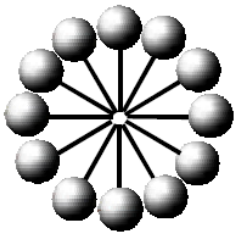

Figure 3 *Micelle*

At low concentrations surfactants will favour arrangement on the surface. As the surface becomes crowded with surfactant more molecules will arrange into micelles. At some concentration the surface becomes completely loaded with surfactant and any further additions must arrange as micelles. It follows that measurement of surface tension may be used to find CMC. The concentration at which this effect occurs is known as the *critical micelle formation concentration* (CMC) in figure (Figure 4) [8]. It is an important characteristic for surfactants. This means that methods for measuring the dynamic surface tensions should only be used above the CMC [12].

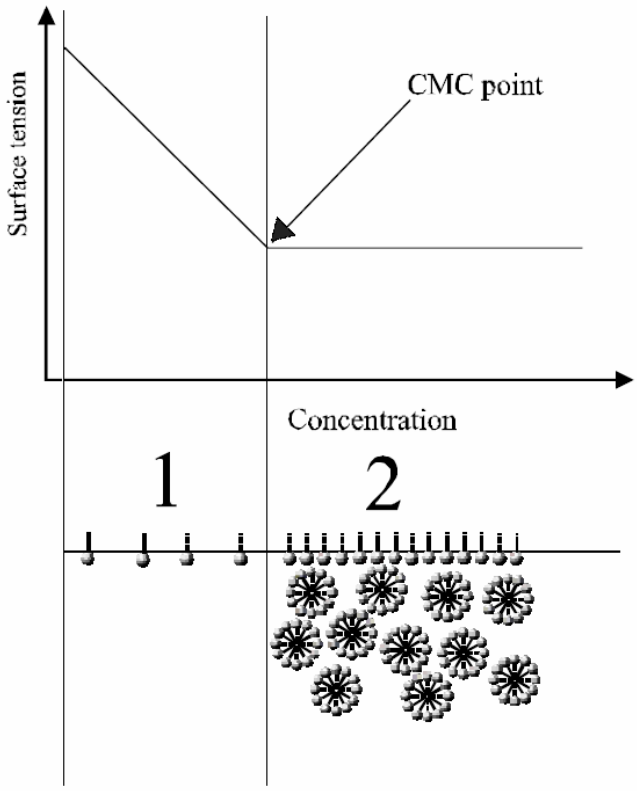

Figure 4 *Critical micelle concentration*

Good example of anionic surfactant with amphiphilic properties is *sodium dodecyl sulphate* (SDS) used in household products. It is probably the most researched surfactant compound and is used as a model for comparison with many amphiphiles. Surface properties (γ), sorption and micellization are compared SDS with hyaluronan.

2.2.1 Hydrophobically modified polymers

Hydrophobically modified polymers (HM polymers) are amphiphilic water-soluble macromolecules mainly constituted of a hydrophilic backbone and hydrophobic side groups. They have unique associative behaviour and they are used in many branches, such as pharmaceuticals, cosmetics, paints, etc [13].

The physicochemical properties of HM polymers depend on structural parameters of the polymer. The nature of the macromolecular backbone, the length and rate of hydrophobic moieties and also on environmental parameters - pH, salinity, temperature, etc. HM biopolymers are e.g. carboxymethylcellulose, hyaluronan, dextran, alginate, carrageenan, starch, or chitosan [13].

Interesting physicochemical characteristics of the hydrophobically modified polymers are related to both intra- and/or intermolecular associations between their hydrophobic segments in aqueous solution, which would lead to the formation of more or less aggregated structures containing hydrophobic microdomains. The conformation of these polymers in solution may be studied by fluorescence techniques using probes such as pyrene [13].

Due to their amphiphilic structure, HM polymers also have potential high surface and interfacial properties. They diffuse through the bulk phase and adsorb at the interface, inducing a sharp reduction in the surface or interfacial tension of a polymer solution [13].

Surface tension studies have evidenced various structures at the air–water interface depending on the concentration and the characteristics of these polymers. Adsorption at the air–water interface strongly depends on macromolecular architecture such as the stiffness and the charge density for polyelectrolytes. The high stiffness of a macromolecular skeleton would limit the number of contacts of hydrophobic moieties with the surface. Also, the chemical nature and the rate and length of the hydrophobic groups would affect the surface activity of these surfactants [13].

Investigation of HM polymers

Surfactant solutions comprising of normal or reverse micelles are used as media for a variety of chemical analysis and synthesis. Normal micelles that form within aqueous surfactant solutions above a surfactant concentration (CMC), are a topic of major interest due to their unusual physicochemical properties as a result of surfactant aggregation [14].

Micellar systems have immense technological applications such as flow field regulators, solubilizing and emulsifying agents, membrane mimetic media, or nanoreactors for enzymatic reactions. At ambient conditions, properties of an aqueous surfactant solution depend on the identity of the surfactant along with its concentration in the solution. One way to alter/modify the physicochemical properties of a given aqueous surfactant solution is to use external means, such as, changes in temperature/pressure and/or addition of a variety of modifiers [14].

Hyaluronic acid

Also called hyaluronan, or hyaluronate exists in the salt form. It is a high molecular weight and linear unbranched polymer. Exists in every vertebral body in skin, eyes, joints, hair and cartilages. It is an mucopolysaccharide composed of D-glucoronic acid and D-N-acetylglucosamine linked together by a glycosidic bonds (Figure 5) [24] and in unbranched chain has up to 10 000 saccharide articles.

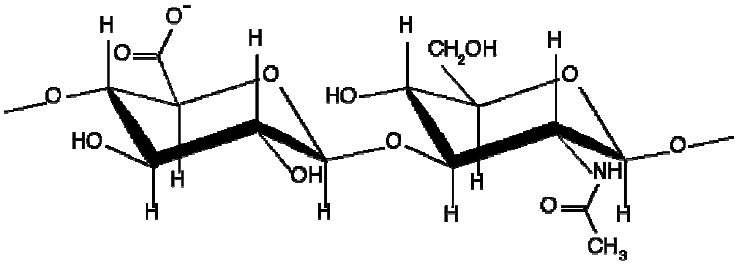

Figure 5 *Hyaluronic acid structure*

Hyaluronic acid has high affinity to water, is able to fix water in volume 100% of his weight and has excellent hydration effects. Water solution of hyaluronic acid is viscoelastic because of the viscose and elastic components.

The structure consists of hydrofobic and hydrofilic parts. The backbone of a hyaluronic acid molecule is a stiffened by a combination of the disaccharide, internal hydrogen bonds and interactions with solvent. The axial hydrogen atoms form a non-polar hydrophobic face, and the equatorial hydrogen atoms form a polar hydrophilic face. They create a twisting ribbon structure [15].

The changes of the carboxylic group of D-glucoronic acid, which are influenced by the ionic strength and pH of the environment, influence the shape of the chains and their interactions with surrounding molecules [16].

The domain structure of hyaluronic acid has important effects. Small molecules (water, electrolytes, nutrients) can freely diffuse through the solvent within the domain. But the large molecules (proteins) are excluded from the domain because of their hydrodynamic size in solution. The hyaluronic acid chains are constantly moving in the solution and the 'pores' in the network continuously change the size. All sizes of this pores can exist, but with different probabilities, and all molecules can pass through the network with different degrees of retardation [15].

Only a few measurements of hyaluronic acid by the maximum bubble pressure were presented in the literature. It is a relatively new situation. There is shown a dependence of sodium hyaluronate (NaHA) in the figure (Figure 6) [18] measured by the pedant drop method. It is equilibrium surface tension of sodium hyaluronate solutions as a function of concentration at 25°C [18]. Surface tension starts to decrease at about concentration $c = 2.5 – 3$ g/l. From $c = 2.5 – 3$ g/l is relatively acute decline. Surface tension decrease from about 70 mN/m to 50 mN/m. Decreasing stops around concentration $c = 3.5$ g/l.

In this work is surface tension of hyaluronic acid and it's derivatives measured by the maximum bubble pressure method.

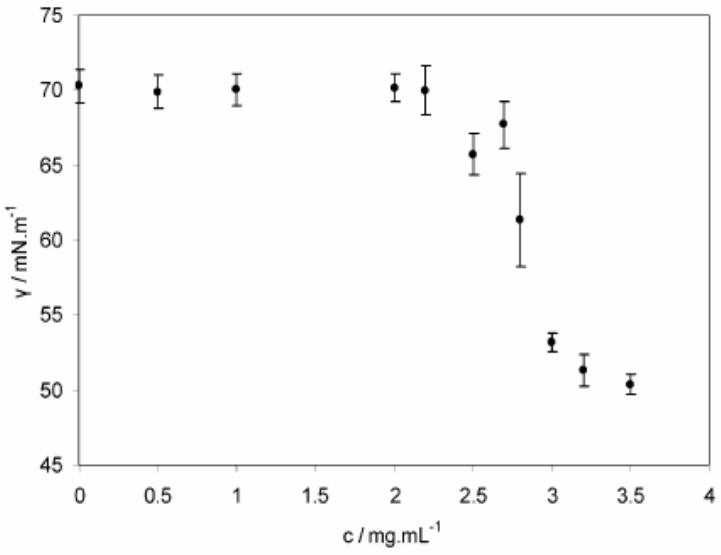

Figure 6 *Surface tension of NaHA*

Sodium dodecyl sulphate

$CH_3(CH_2)_{10}CH_2\text{-}SO_4^-\ Na^+$ as shown in the figure (Figure 7) [25] is an anionic surfactant, which is used in household products. It is a typical anionic surfactant that has been used widely as a hydrate promoter. The interfacial tension between gas and liquid is strongly dependent on the properties of the liquid phase [19].

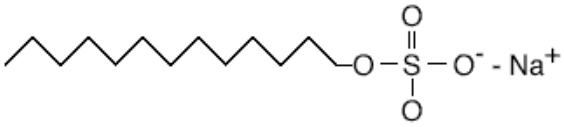

Figure 7 *Sodium dodecyl sulphate*

Many journals have been published on the different properties of aqueous solution of SDS through different approaches [19]. In the figure (Figure 8) [17] is the sodium dodecyl sulphate surface tension in 0.1 M NaCl measured by the maximum bubble pressure method. Surface tension decrease with increasing concentration of SDS solution [17].

In this work is SDS used as a model measurement for examination of device functions and measurability and for hyaluronic acid and its derivatives comparison.

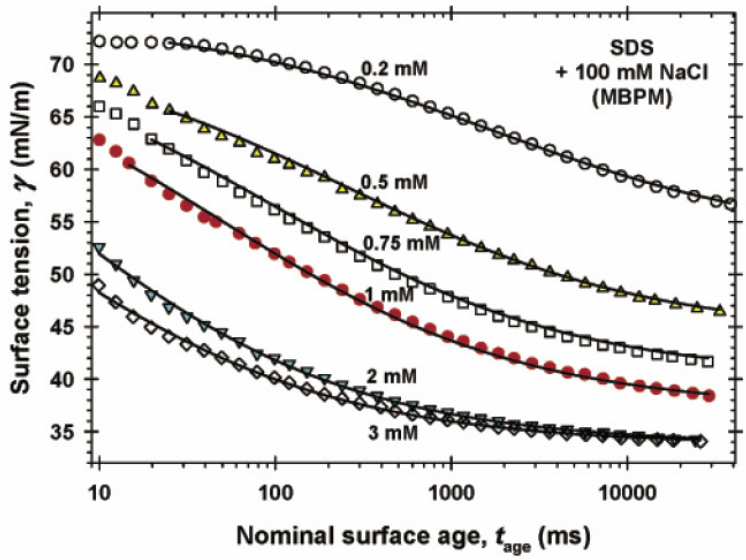

Figure 8 *Dynamic Surface tension of SDS in 0.1 M NaCl*

3 EXPERIMENTAL PART

3.1 Materials

Hyaluronic acid (HA) was provided by Contipro Group s.r.o. from Dolni Dobrouc Czech republic in two molecular mass: Mw = 0.4645 MDa and Mw = 1.69 MDa. Derivatives *C8-NH-HA-9* (octyl) with Mw = 418.4 kDa, *C10-NH-HA-30* (decyl) with Mw = 43.28 kDa, *C10-NH-HA-50* (decyl) with Mw = 45.46 kDa also from Contipro Group s.r.o.

Another used adducts *sodium dodecyl sulphate* (SDS) with Mw = 288.38 g/mol, sterile water for injection (Fresenius, Italy), *sodium chloride* (NaCl) Mw = 54.44 g/mol, *sodium iodide* (NaI) Mw = 149.89 g/mol, *sodium bromide* (NaBr) Mw = 102.89 g/mol, *sodium sulphate decahydrate* ($Na_2SO_4 \cdot 10H_2O$) Mw = 322.19 g/mol, *sodium perchlorate monohydrate* ($NaClO_4 \cdot H_2O$) Mw = 154.45 g/mol.

3.2 Methods

At first the density of samples was measured than viscosity and finally the surface tension.

3.2.1 Density

Density of the samples was measured with densitometer Densito 30PX at laboratory temperature. Densities of all samples differed only slightly. First and the last one sample in each series were measured. If there were some bigger difference the whole series was measured by the densitometer.

3.2.2 Viscosity

Viscosity was measured by the Anton Paar AMVn Automated Microviscometer. All samples were measured at 23°C with estimated density before. Each sample was measured by two methods. Once with 50° angle inclination of capillary and once with 70° angle inclination.

3.2.3 Surface tension

For surface tension measuring was used the maximum bubble pressure tensiometer BPA-800P from KSV Instruments (Finland) connected with PC. The used capillary has 0.13 mm radius and 0.5 mm immersion depth. All samples were measured at laboratory temperature because there is no possibility of temperature control.

3.3 Preparation and measuring of HA (Mw = 0.4645 MDa)

This is a model information about preparation and measuring of samples. There were 16 concentration series of samples prepared, for simplicity is subsequently shown only the first concentration series of Hyaluronic acid with Mw = 0.4645 MDa in 0.15 M NaCl.

50g of stock solution with concentration 5g/l was prepared from hyaluronic acid (HA) and water for injection. HA (Mw = 0.4645 MDa) was slowly added into the water on working

stirring arrangement. Stock solution was mixed for 3 days until the HA was completely melted.

Sodium hyaluronate solutions were prepared in two series from stock solution. Hyaluronic acid in water for injection (water) and hyaluronic acid in water + sodium chloride (NaCl) with concentration 0.3 M. Both concentrations of series were between 0.1 g/l and 2 g/l.

For first series of solutions (HA in water) was used a middle of stock solution (SS). Single samples were prepared on analytical scale. Into the beaker was added calculated amount of SS and sample was completed with water. Total amount of samples were 15 g.

For second series of solutions (HA in NaCl) was used a second half of stock solution (SS). Single samples were prepared also on analytical scale. Into the beaker was added calculated amount of SS, than completed with water (w) into 7.5 g and at least was sample completed with NaCl. Total amounts of samples were also 15 g. Both series (16 samples) were put on the stirring arrangement for 3 hours. Accurate amounts of components are in the table (Table 1).

Table 1 *Amounts of components in samples (*w *– water)*

No.	c(g/l)	HA in water (g)		HA in NaCl (g)		
		m(HA)	m(HA+w)	m(HA)	m(HA+w)	m(HA+w+NaCl)
1	0.1	0.3094	15.0030	0.3308	7.5159	15.0062
2	0.2	0.6007	15.0038	0.6218	7.5353	15.0102
3	0.5	1.4982	15.0184	1.5207	7.5376	15.0394
4	0.7	2.1320	15.0319	2.1021	7.5012	15.0188
5	1	3.0257	15.0231	3.0154	7.5404	15.0176
6	1.25	3.7686	15.0969	3.7730	7.5474	15.0188
7	1.5	4.4734	15.0303	4.5894	7.5468	15.0035
8	2	6.0495	15.0309	6.0280	7.5189	15.0095

After the mixing of samples the measurements started. At first was measured the density of the least and the most concentrated samples in each row at the room temperature. This measurement was only for control because there weren't expected any big density differences. For details see values in the table (Table 2). Density was necessary to insert to viscosity device for next measuring of viscosity. After this can be started the viscosity measurement at 23°C temperature with given density. Each sample was measured two times for control. Once with 50° angle inclination of capillary and once with 70° angle inclination. The values of viscosity in mN/m are in the table (Table 2).

Table 2 *Viscosities and density of samples*

No.	c(g/l)	Density (g/cm^3)	HA in water (mPa·s)			HA in NaCl (mPa·s)		
			angle 70°	angle 50°	average	angle 70°	angle 50°	average
1	0.1052	1.006	1.8045	1.8779	1.8412	0.9512	0.9436	0.9474
2	0.2043	1.006	2.5489	2.6874	2.6182	0.9929	0.9837	0.9883
3	0.5089	1.006	4.4173	4.6099	4.5136	1.2210	1.2157	1.2184
4	0.7236	1.006	4.9452	5.0092	4.9772	1.3944	1.3856	1.3900
5	1.0275	1.006	6.3867	6.5089	6.4478	1.6953	1.6937	1.6945
6	1.2735	1.006	7.4326	7.7147	7.5737	1.9717	1.9612	1.9665
7	1.5184	1.006	8.8598	9.0752	8.9675	2.2834	2.2847	2.2841
8	2.0533	1.006	11.2576	11.692	11.4748	3.2156	3.4018	3.3087

Obtaining of the viscosity values finally led to measuring of surface tension. For each sample was set the concentration, density and the average value of both viscosities (with 50° and 70° angle inclination). Each sample was measured twice. Firstly with *standard* method and second with *increasing flow rate* method. Both at the room temperature.

In the table (Table 3) are measured values by the tensiometer. In the first column there is a gas pressure *P* (Pa) in a bubble. Next column *Q* (mm^3/s) represents the flow gas amount. In standard method flows the gas gradually down. *T life* is the life time of the bubble. Next two columns *Sqrt(Tlife)* and *1 / Sqrt(Tlife)* are converted from *T life*. *ST* (mN/m) is surface tension.

Table 3 *Output data from device*

Measuring of HA (Mw = 0.4645 MDa) in NaCl Standard method - T life = 10s c = 0.1 g/l					
P (Pa)	Q (mm^3/s)	T life	Sqrt(Tlife)	1 / Sqrt(Tlife)	ST (mN/m)
1183.4	77.5	0.0172	0.1311	7.6249	73.89
1173.4	70.4	0.0232	0.1523	6.5653	73.38
1165.1	62.4	0.0305	0.1746	5.7260	72.95
1160.4	56.2	0.0388	0.1970	5.0767	72.71
1157.3	50.8	0.0499	0.2234	4.4766	72.57
1155.6	44.9	0.0643	0.2536	3.9436	72.51
1153.4	36.5	0.0835	0.2890	3.4606	72.41
1151.3	30.9	0.1130	0.3362	2.9748	72.30
1149.3	25.8	0.1437	0.3791	2.6380	72.19
1148.1	21.0	0.1787	0.4227	2.3656	72.13
1146.2	15.8	0.2556	0.5056	1.9780	72.03
1146.4	11.3	0.3112	0.5579	1.7926	72.06
1144.2	10.9	0.3803	0.6167	1.6216	71.92
1143.8	7.6	0.4743	0.6887	1.4520	71.91

1143.5	5.9	0.6009	0.7752	1.2900	71.91
1141.8	0	0.8780	0.9370	1.0672	71.83
1141.9	0	1.0800	1.0392	0.9623	71.90
1139.9	0	1.3795	1.1745	0.8514	71.77
1141.5	0	1.8215	1.3496	0.7409	71.87
1141.5	0	2.3690	1.5392	0.6497	71.87
1140.7	0	3.0325	1.7414	0.5742	71.82
1135.4	0	4.6770	2.1626	0.4624	71.47
1139.2	0	6.2005	2.4901	0.4016	71.72
1136.1	0	7.6410	2.7642	0.3618	71.52
1135.4	0	16.5375	4.0666	0.2459	71.47

From these output data is necessary to calculate complete next data in the table (Table 4). The BPA software does not copy temperatures and time, that's why it was also completed. Tensiometer can't keep constant temperature so it was necessary calculate surface tension data on water according to correction equation [6]:

$$\gamma = 75{,}621 - 0{,}15 \cdot t - 1{,}0266 \cdot 10^{-4} \cdot t^2 , \tag{5}$$

where t is the temperature of measured sample. After this correction were obtained new surface tension values. Every measurement provided about 30 values of surface tension depending on time life. To obtaining one equilibrium surface tension was used a special equation [6]:

$$\gamma = \gamma_{eq} + \frac{s_\gamma}{a_\gamma + t_{age}^{\,1/2}} , \tag{6}$$

where t_{age} and the parameters s_γ and a_γ are orientation values calculated from another complicated equations, but for simplification were used these orientation values from measured experiments according to journal [17]. Final equilibrium surface tension γ_{eq} as shown in the table (Table 4) is red marked.

Table 4 *Completed and calculated data*

Measuring of HA (Mw = 0.4645 MDa) in NaCl Standard method - T life = 10s c = 0.1 g/l								
t (sec)	T(°C)	correction	/1000	Ds(m^2/s)	model ST (mN/m)	sumx2y2	γ_{eq}	**72.761**
							s_γ	0.4651
96	23.7	75.0374	0.0750	1.0284	73.7320	$1.82 \cdot 10^{-6}$	a_γ	0.4617
121	23.7	74.5194	0.0745	0.7519	73.7200			
174	23.6	74.0828	0.0741	0.5653	73.7060			
215	23.6	73.8390	0.0738	0.4414	73.6900			
250	23.5	73.6969	0.0737	0.3419	73.6700			
292	23.5	73.6359	0.0736	0.2649	73.6450			
327	23.5	73.5344	0.0735	0.2034	73.6140			

352	23.4	73.4227	0.0734	0.1499	73.5700
397	23.4	73.3110	0.0733	0.1175	73.5290
439	23.4	73.2500	0.0733	0.0943	73.4870
484	23.3	73.1485	0.0731	0.0658	73.4090
522	23.3	73.1790	0.0732	0.0541	73.3630
562	23.3	73.0368	0.0730	0.0441	73.3130
600	23.3	73.0266	0.0730	0.0353	73.2580
642	23.3	73.0266	0.0730	0.0279	73.1990
676	23.3	72.9454	0.0729	0.0190	73.1080
711	23.3	73.0165	0.0730	0.0155	73.0630
748	23.2	72.8844	0.0729	0.0121	73.0140
773	23.2	72.9860	0.0730	0.0092	72.9650
801	23.2	72.9860	0.0730	0.0071	72.9250
831	23.2	72.9352	0.0729	0.0055	72.8940
877	23.2	72.5798	0.0726	0.0035	72.8510
914	23.2	72.8337	0.0728	0.0027	72.8310
947	23.2	72.6306	0.0726	0.0022	72.8180
980	23.2	72.5798	0.0726	0.0010	72.7880
	23.36 – temperature average				

Evaluation of all data was make in MS Excel by the help of solver which found a graphical model of curve and calculated above mentioned equilibrium surface tension.

For each experiment was calculated equilibrium surface tension and made two graphs dependence of surface tension on T life and 1/Sqrt (T life). As shown in figures (Figure 9 and Figure 10) the experimental measured data are the black dots and have decreasing tendency. For obtaining equilibrium surface tension solver made a curve and calculate the equilibrium surface tension value.

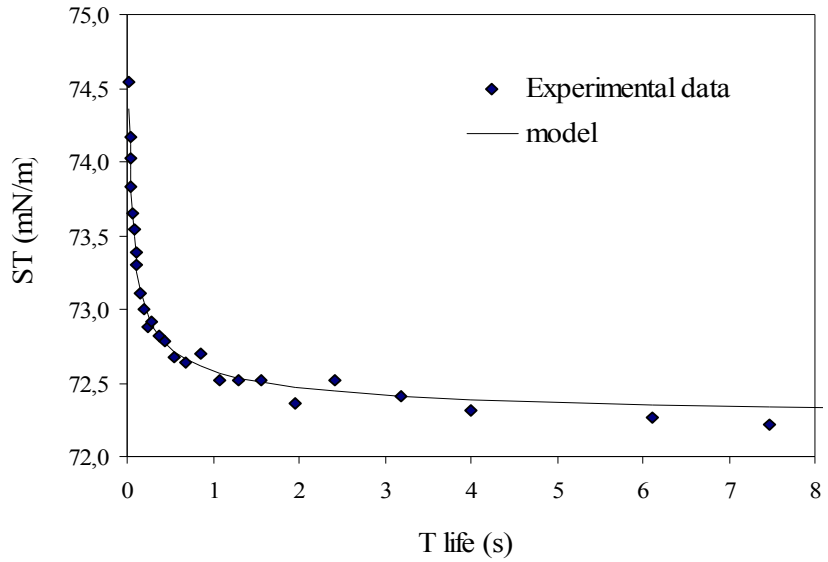

Figure 9 *Insert of model curve in experimental data ST vs. T life*

For controlling right functioning of the model was make another graph showing dependence of surface tension on 1/Sqrt (T life). Experimental data are also the black dots and solver also completed the line model. As shown in figures (Figure 9, Figure 10) this method of data evaluation seems to be suitable.

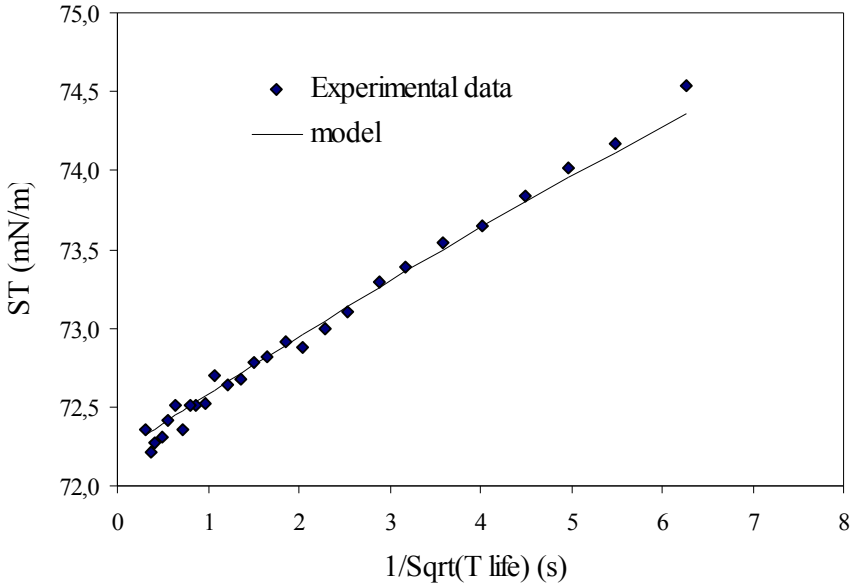

Figure 10 *Insert of model curve in experimental data ST vs. 1/Sqrt(T life)*

There were 16 series and each series had about 10 samples with increasing concentration. Calculation as was remarked above was made for each sample in each series. Finally there were about 20 equilibrium surface tension values in each concentration series because each sample was measured two times – with standard and increase flow rate (IFR) methods. For simplicity are in next parts shown only tables with concentrations and equilibrium surface tension values (for standard and IFR method averaged) and final graphs with dependence of equilibrium ST on logarithm concentration – log c.

For each experiment was also calculated diffusion constant D_s [m^2/s] from equation [26]

$$\gamma_t = \gamma_0 - 2RTc \cdot \sqrt{\frac{D_s t}{\pi}}, \tag{7}$$

and made a graph (Figure 11).

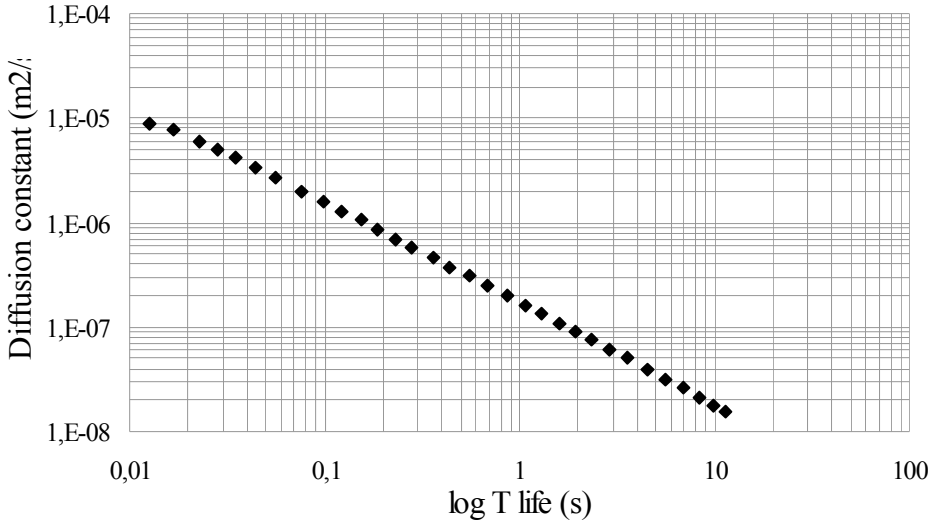

Figure 11 *Dependence of diffusion constant on log T life*

4 RESULTS AND DISSCUSION

There were measured a lot of experiments so each experiment was measured by the standard and increase flow rate methods. The methods are nearly the same so there was established an error for each experiment which was within the range of 0.01 - 5 %.

At first were the single samples from each concentration series studied. In the figure (Figure12) are investigated surfactants shown. From each surfactant was one sample chosen. All of them were in NaCl measured. Concentrations of samples in NaCl are nearly the same except lower concentration of SDS because of the comparison surface tension range in figure (Figure12). Apparently hyaluronic acid doesn't show some big decrease compared to derivatives and SDS. The greatest decrease of HA is shown at the top of the measuring and than follow almost no decrease. Whereas derivatives and SDS show almost constant decrease during the whole measuring.

This different process is caused by the different adsorption and diffusion velocity of various molecules on the bubble surface. Some molecules, especially the smaller, adsorb themselves on the surface very quickly. So this could be the answer of very quick decrease of surface tension on the beginning of measuring. Whereas bigger molecules need longer time for adsorption so than they evoke next curve decrease. Therefore each molecule has a different adsorption and diffusion velocity.

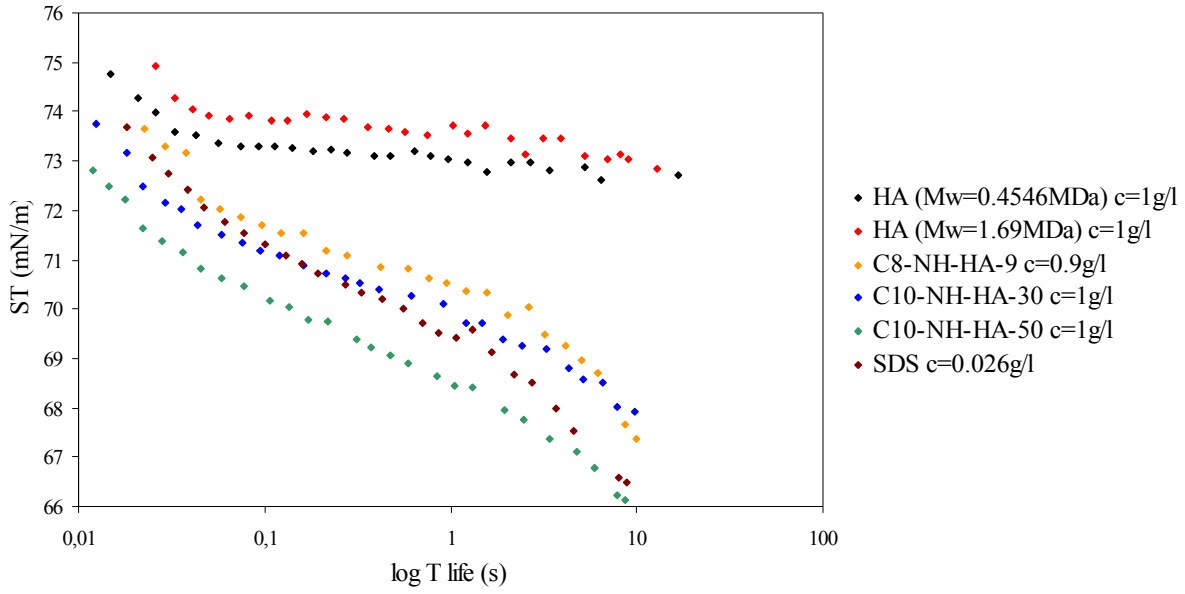

Figure12 *Dynamic surface tension vs. log T life of measured samples*

4.1 Sodium dodecyl sulphate

Sodium dodecyl sulphate was measured in water and in 0.15 M NaCl. Standard method and IFR method were used. Many journals have been written about this surfactant, so this was used as an model how does the device work and for comparison with other measured surfactants.

Surface tension of SDS measured in water and NaCl goes rapidly down in the figure (Figure 13). Values of equilibrium surface tension are summarized in the table (Table 5). SDS in NaCl surface tension decrease starts earlier than SDS in water. Ionic surfactant SDS has repulsive SO^{3-}. After NaCl addition Na^{+} goes to SO^{3-} and detachment forces are compensated. Faster aggregation causes earlier surface tension decrease. The difference between surface tension of first and last concentration is around 34 mN/m.

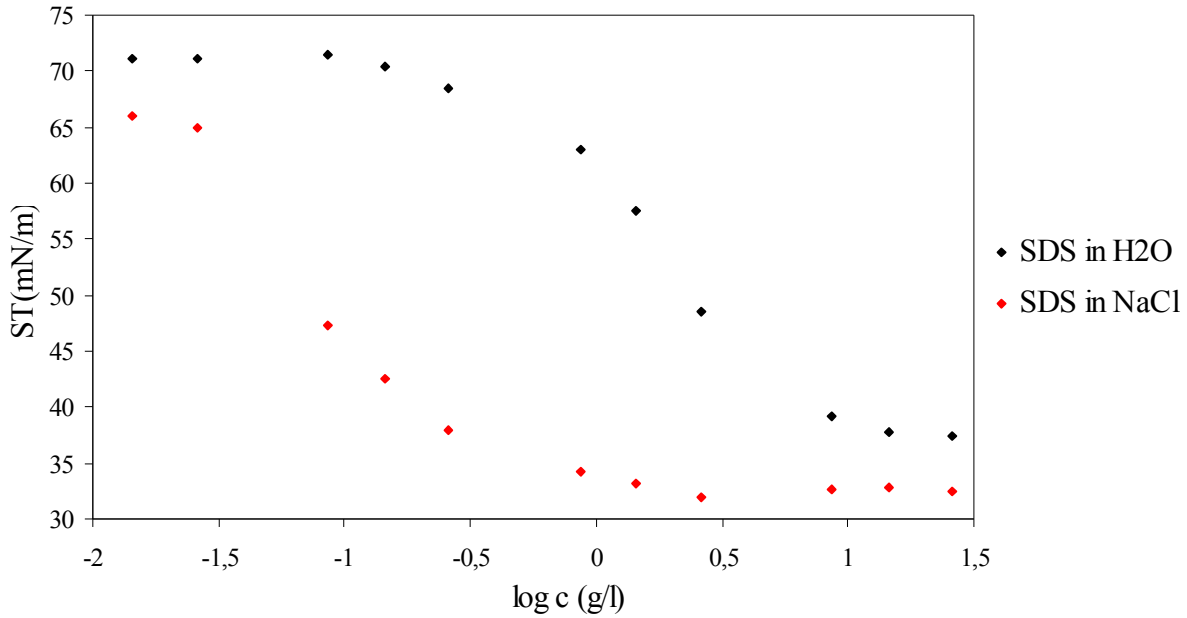

Figure 13 *Dynamic ST of SDS in H_2O and in NaCl dependence on log c*

Table 5 *Equilibrium surface tension of SDS*

theoretic c(g/l)	c (mol/l)	ST (SDS in water) mN/m	ST (SDS in NaCl) mN/m
0.0144	$0.5 \cdot 10^{-4}$	71.1814	66.0001
0.0260	$0.9 \cdot 10^{-4}$	71.1328	64.8592
0.0865	$0.3 \cdot 10^{-3}$	71.3894	47.2387
0.1442	$0.5 \cdot 10^{-3}$	70.4642	42.5091
0.2595	$0.9 \cdot 10^{-3}$	68.4844	37.9192
0.8651	$0.3 \cdot 10^{-2}$	63.0565	34.3010
1.4419	$0.5 \cdot 10^{-2}$	57.4460	33.2213
2.5954	$0.9 \cdot 10^{-2}$	48.5189	32.0203
8.6514	$0.3 \cdot 10^{-1}$	39.1576	32.6374
14.4190	$0.5 \cdot 10^{-1}$	37.6789	32.8279
25.9542	$0.9 \cdot 10^{-1}$	37.3923	32.4780

4.2 Hyaluronic acid (Mw = 0.4645 MDa, Mw = 1.69 MDa)

Hyaluronic acid was studied in two solutions. In water and 0.15 M NaCl. There were used standard and increase flow rate methods. Each solution was measured by these two methods.

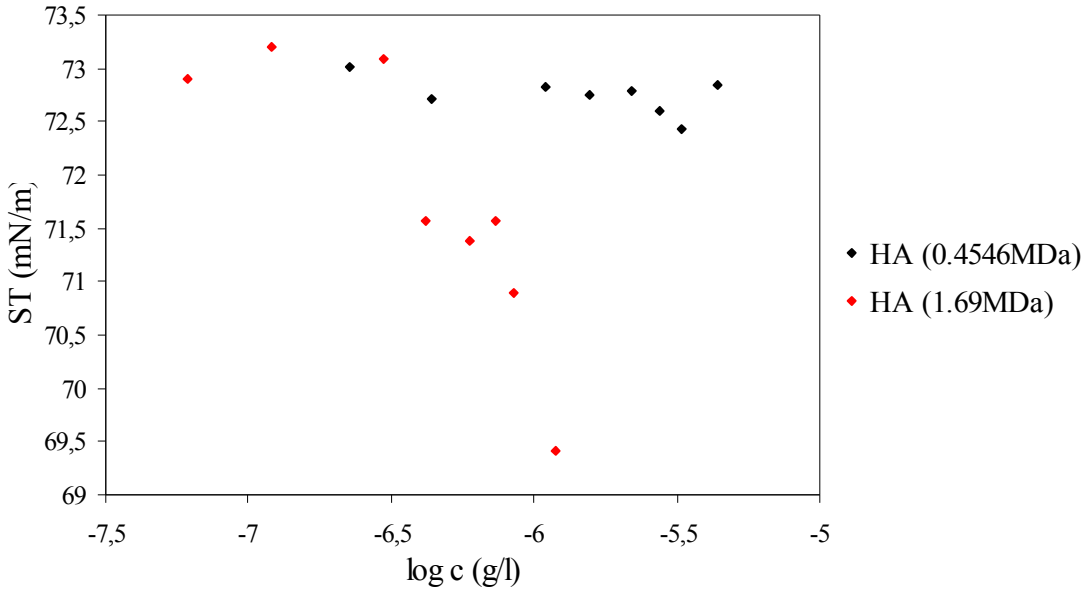

Figure 14 *Hyaluronic acid in water, ST vs. log c*

Hyaluronic acid in water shows only minimum decrease in range 4 mN/m from value 69.4 – 73.2 mN/m in the figure (Figure 14). Equilibrium surface data in standard and increase flow rate methods are rather different and so could be said that solution has no surface activity. Values of surface tension are summarized in the table (Table 6). This small decrease can be also at the level of error.

Table 6 *Values of equilibrium surface tension of HA in water*

theoretic c(g/l)	ST (0.4546MDa) mN/m	ST (1.69MDa) mN/m
0.1	73.0201	72.8955
0.2	72.7141	73.2003
0.5	72.8229	73.0844
0.7	72.7560	71.5726
1.0	72.7915	71.3824
1.2	72.5992	71.5598
1.5	72.4289	70.8923
2.0	72.8382	69.4200

0.15 M NaCl was added to hyaluronic acid and measured. Also this case shows no surface activity as shown in the figure (Figure 15). The range of decrease is around 2 mN/m from 73.5 - 71.2 mN/m. This decrease is minimal. Values of surface tension are in the table (Table 7). To the future measuring is necessary to try higher concentrations of hyaluronic acid.

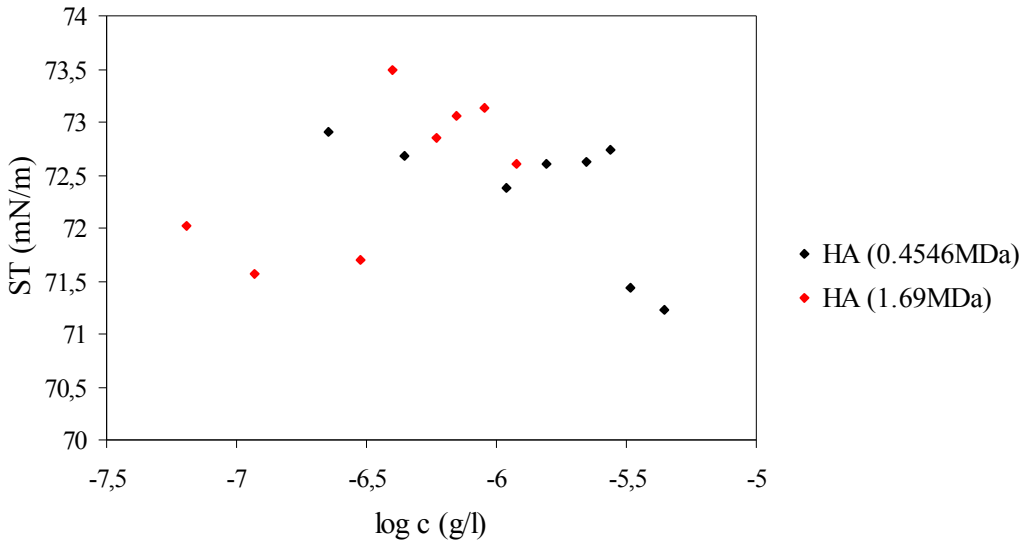

Figure 15 *Hyaluronic acid in NaCl, ST vs. log c*

Table 7 *Values of equilibrium surface tension of HA in NaCl*

theoretic c(g/l)	ST(0.4645MDa) mN/m	ST (1.69MDa) mN/m
0.1	72.9111	72.0133
0.2	72.6783	71.5614
0.5	72.3715	71.6941
0.7	72.5956	73.4887
1.0	72.6284	72.8533
1.2	72.7303	73.0474
1.5	71.4259	73.1405
2.0	71.2345	72.6024

More recapitulation of measuring is needed for the next investigation of pure hyaluronic acid. There is no surface activity in this concentration range. If there will by some future measurements of hyaluronic acid, higher concentrations are wanted. There is about 3 g/l and higher concentration investigated [18]. But there is another problem with liquidity. Until the concentration about 3 g/l is HA relatively liquid but with increasing concentration this liquidity decrease. In higher concentrations hyaluronic acid became a gel and it's not a surfactant. Than we speak about surface energy, not the surface tension, and the maximum bubble pressure method can't be used.

4.3 Derivate C8-NH-HA-9 in H_2O, 0.01 M NaCl, 0.15 M NaCl, 0.5 M NaCl

C8-NH-HA-9 the derivate of hyaluronic acid was measured in water, in 0.15 M NaCl, 0.01 M NaCl and in 0.5 M NaCl. Solutions were prepared in different concentration ranges see in the table (Table 8). In the figure (Figure 16) four derivatives are compared. C8-NH-HA-9 in 0.5 M NaCl shows highest surface tension values. Na^+ are bonded to molecule and it causes higher ion strange in solution. Surface tension C8-NH-HA-9 in water shows decrease as first with C8-NH-HA-9 in weak 0.01 M NaCl. Next decrease of derivatives in NaCl is expected and wider concentration range of these derivatives in NaCl is in future measurement needed.

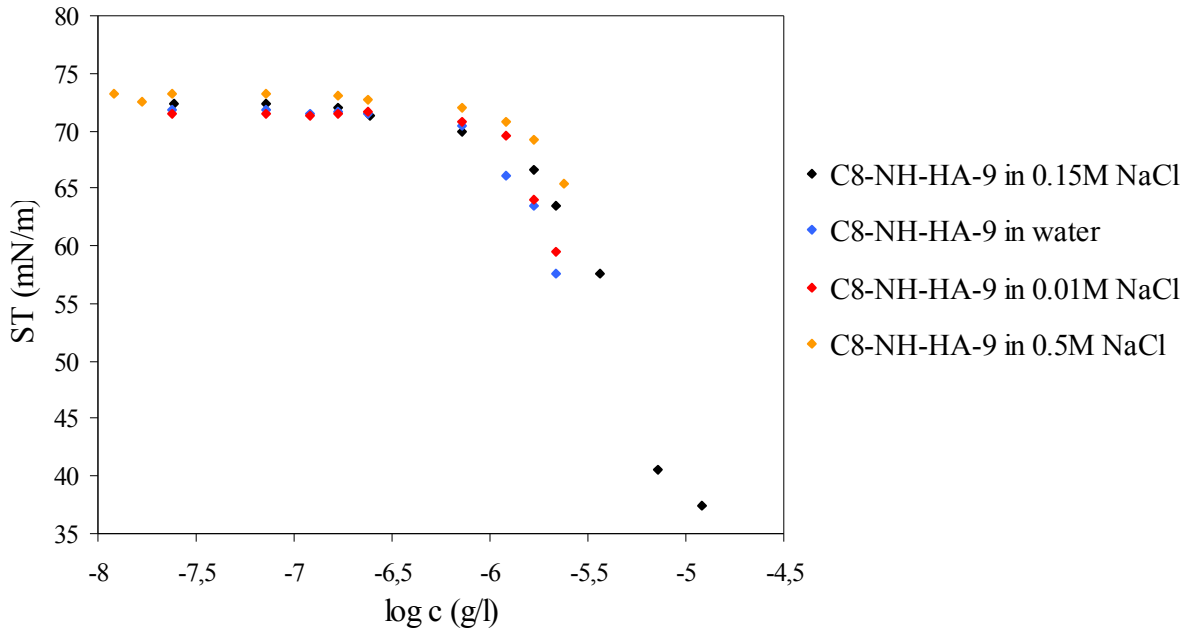

Figure 16 *Surface tension of derivative in NaCl and water*

Table 8 *Surface tension values of derivative in various concentrated NaCl and in water*

theoretic c(g/l)	der. in 0.15 M NaCl (mN/m)	theoretic c(g/l)	der. in water (mN/m)	der. in 0.01 M NaCl (mN/m)	theoretic c(g/l)	der. in 0.5 M NaCl (mN/m)
0.01	72.3502	0.01	71.7739	71.4001	0.005	73.1444
0.03	72.3002	0.03	71.7652	71.4220	0.005	72.5508
0.07	72.0804	0.05	71.4144	71.2706	0.01	73.1843
0.1	71.2751	0.07	71.7293	71.5407	0.03	73.1720
0.3	69.8684	0.1	71.4522	71.6203	0.07	72.9974
0.7	66.5562	0.3	70.3776	70.7346	0.1	72.7874
0.9	63.4837	0.5	66.0306	69.4960	0.3	72.0233
1.5	57.5204	0.7	63.5226	64.0446	0.5	70.8340
3	40.4925	0.9	57.5090	59.5586	0.7	69.2279
5	37.5008				1	65.4319

There were measured three samples in continual time for better visibility, if there is some change in surface tension during the long time. As shown in the figure (Figure 17), there is one sample of them C8-NH-HA-9 in 0.5 M NaCl with concentration c = 0.07 g/l. Experiment ran for two hours, and the surface tension was during this long time still constant. So there are no changes of surface tension during the long time.

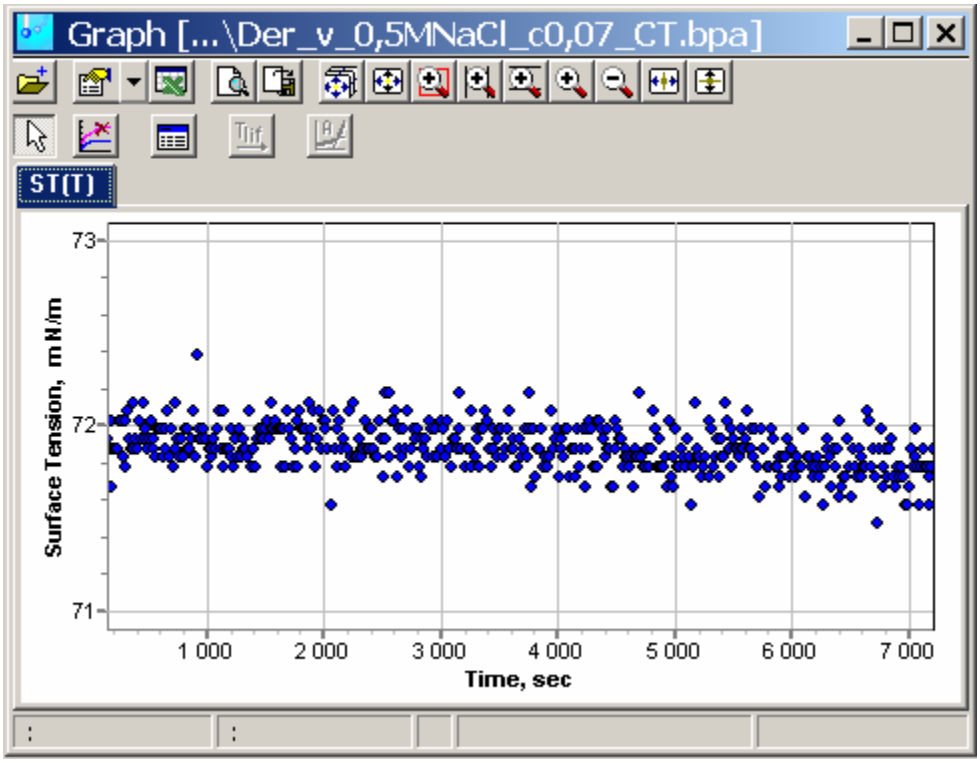

Figure 17 *Measuring of surface tension of C8-NH-HA-9 in 0.5 M NaCl*

4.4 Salts influence comparison

Derivate C8-NH-HA-9 was measured in 0.15M NaCl, NaBr, NaI, Na_2SO_4 and $NaClO_4$, and compared in the figure (Figure 18). Derivate in NaCl shows the biggest decrease. It starts on concentration c = -6 g/l, because this derivative was measured in wide concentration range. Previous measurements of derivatives in NaBr, NaI, Na_2SO_4 and $NaClO_4$, also start to decrease and next decrease continuation is supposed. For future measuring is wider concentration range of derivative necessary to prepare. In the table (Table 9) are summarized dynamic surface tension values.

Table 9 *Dynamic surface tension of derivatives in various salts*

theoretic c(g/l)	ST (NaCl) (mN/m)	theoretic c(g/l)	ST (NaI) (mN/m)	ST (NaBr) (mN/m)	ST (Na_2SO_4) (mN/m)	ST ($NaClO_4$) (mN/m)
0.01	72.3502	0.005	76.0538	72.3719	72.4296	74.5038
0.03	72.3002	0.007	75.6564	72.6976	72.5342	74.4523
0.07	72.0804	0.01	75.3577	72.6688	72.5864	73.8013
0.1	71.2751	0.03	73.5055	72.6407	72.5415	73.9492
0.3	69.8684	0.07	73.1585	72.2484	72.0899	73.5493
0.7	66.5562	0.1	73.1538	72.0965	72.2069	73.8985
0.9	63.4837	0.3	72.6950	71.5045	71.0473	73.0982
1.5	57.5204	0.5	72.0288	70.8773	68.5287	72.6411
3	40.4925	0.7	71.7071	69.6845	65.3090	71.6414
5	37.5008	1	71.1444	68.0710	61.7111	70.7989

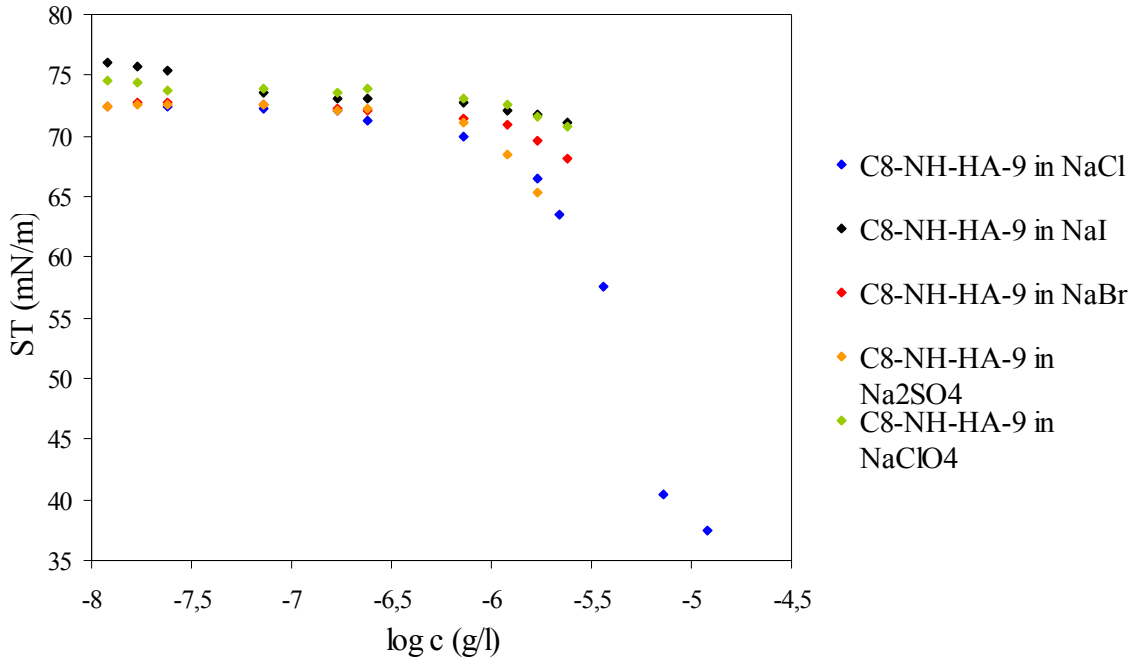

Figure 18 *ST comparison of C8-NH-HA-9 in 0.15M NaCl, NaI, NaBr, Na_2SO_4, $NaClO_4$ on log c*

4.5 Derivatives in 0.15 M NaCl

Derivatives C8-NH-HA-9, C10-NH-HA-30, C10-NH-HA-50 were measured in 0.15 M NaCl and compared in the figure (Figure 19). Surface tension of all derivatives in 0.15 M NaCl starts to decrease on concentration around log c = -6 g/l. Derivatives C10-NH-HA-30 and C10-NH-HA-50 don't show the end of decrease like C8-NH-HA-9 and for future measuring of these derivatives is wider concentration range needed.

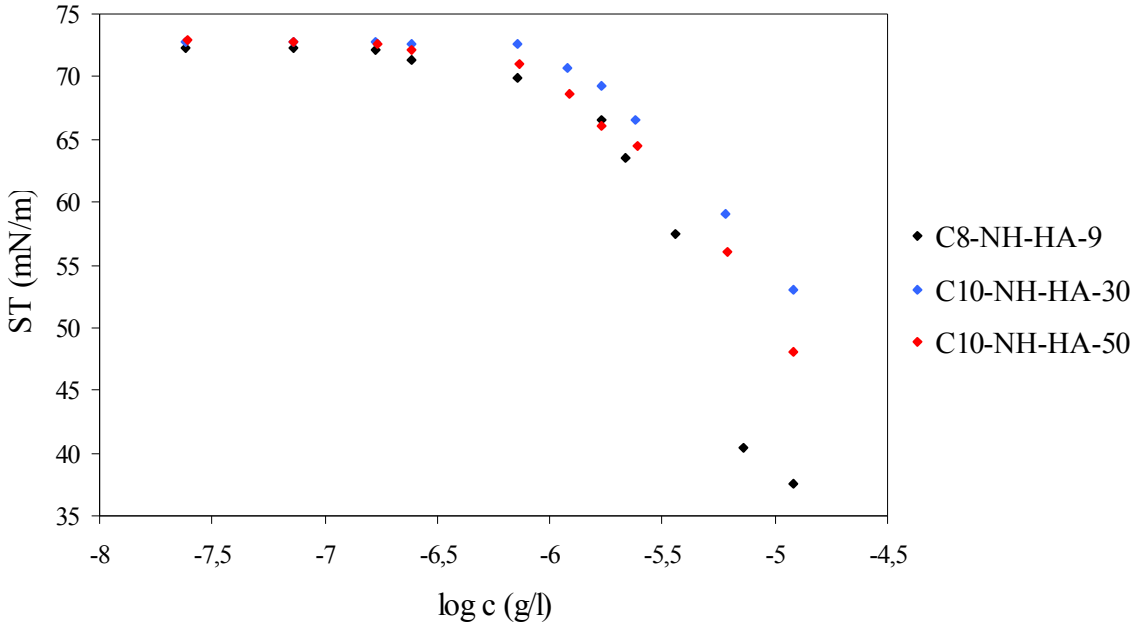

Figure 19 *ST of derivatives in 0.15 M NaCl*

Derivatives C10-NH-HA-30 and C10-NH-HA-50 have higher substitution degree and this could be the reason of higher surface tension values than derivate C8-NH-HA-9 in the table (Table 10).

Table 10 *Dynamic surface tension of derivatives*

theoretic c(g/l)	ST C8-NH-HA-9 (mN/m)	ST C10-NH-HA-30 (mN/m)	ST C10-NH-HA-50 (mN/m)
0.01	72.3502	72.7064	72.9750
0.03	72.3002	72.7404	72.7863
0.07	72.0804	72.7889	72.6488
0.1	71.2751	72.6733	72.1915
0.3	69.8684	72.6194	71.0665
0.7	66.5562	70.6764	68.6108
0.9	63.4837	69.3067	65.9967
1.5	57.5204	66.5253	64.5173
3	40.4925	59.0589	56.0058
5	37.5008	53.0212	48.0106

4.6 Surface tension dynamics

Surface tension dynamics is during the measurement in sample changing. For each concentration series is it a slightly different. At the beginning of the concentration series, where is a low concentration is the dynamic curve relatively the same for all series in example of derivative C8-NH-HA-9 in 0.15 M NaCl c = 0.01 g/l in the figure (Figure 20). Surface tension decrease is only in 3 mN/m range. During the 30 minutes durative measurement was quite continual decrease of surface tension observed. This model was observed in all concentration series respectively in low concetrations of samples.

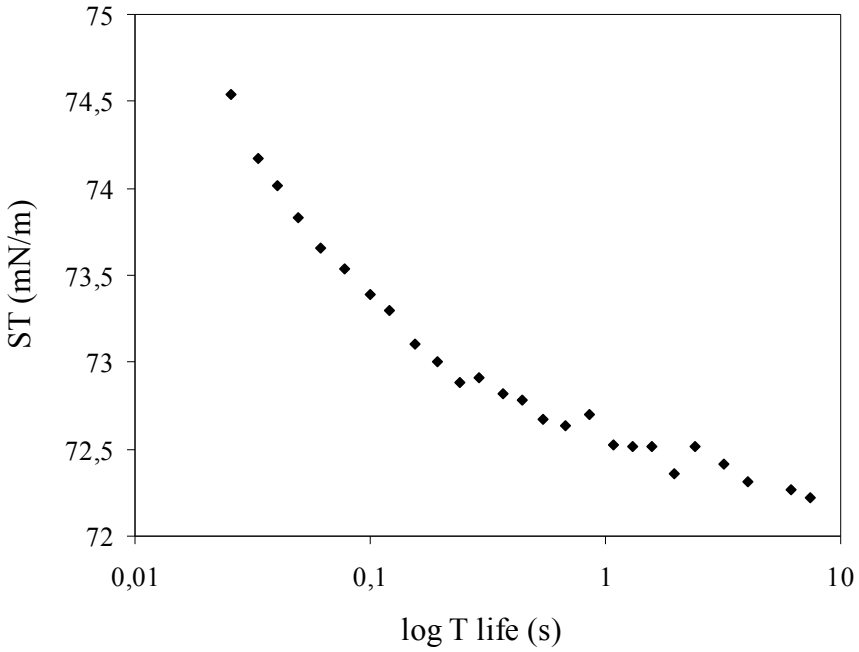

Figure 20 *ST vs. T life of C8-NH-HA-9 in 0.15 M NaCl c = 0.01 g/l*

In higher concentrations of samples – at the end of concentration series – was the dynamics different. On the figure (Figure 21) is for example derivative C8-NH-HA-9 in 0.15 M NaCl c = 5 g/l. Surface tension dynamic curve has very dissimilar running than the example before. The surface tension range is much bigger – around 30mN/m.

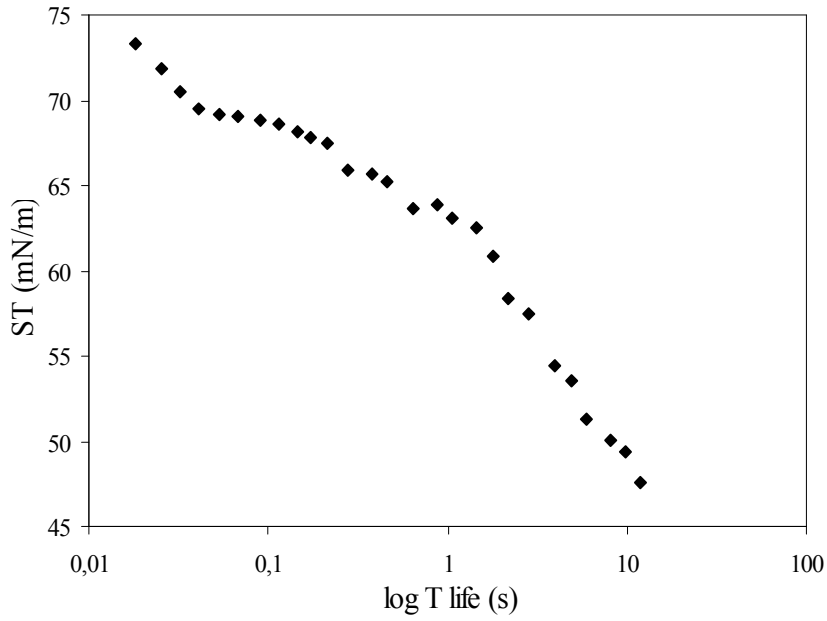

Figure 21 *ST vs. T life of C8-NH-HA-9 in 0.15 M NaCl c = 5 g/l*

Other derivatives C8-NH-HA-9 in 0.15 M NaBr, NaI, Na_2SO_4 and $NaClO_4$ with c = 1 g/l is the dynamics running relatively the same in the figure (Figure 22). It could be said that all measured salts have a similar influence.

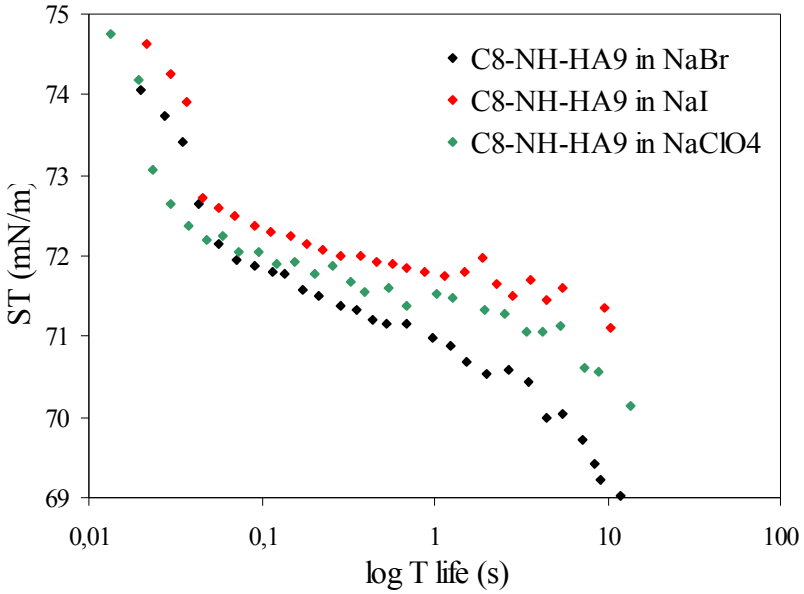

Figure 22 *ST vs. T life of C8-NH-HA-9 in NaBr, NaI, $NaClO_4$*

Another interesting comparison is hyaluronic acid in water and derivate C8-NH-HA-9 in water. Hyaluronic acid shows in high concentration 2 g/l low decreasing running compared to derivative with c = 0.9 g/l in the figure (Figure 23).

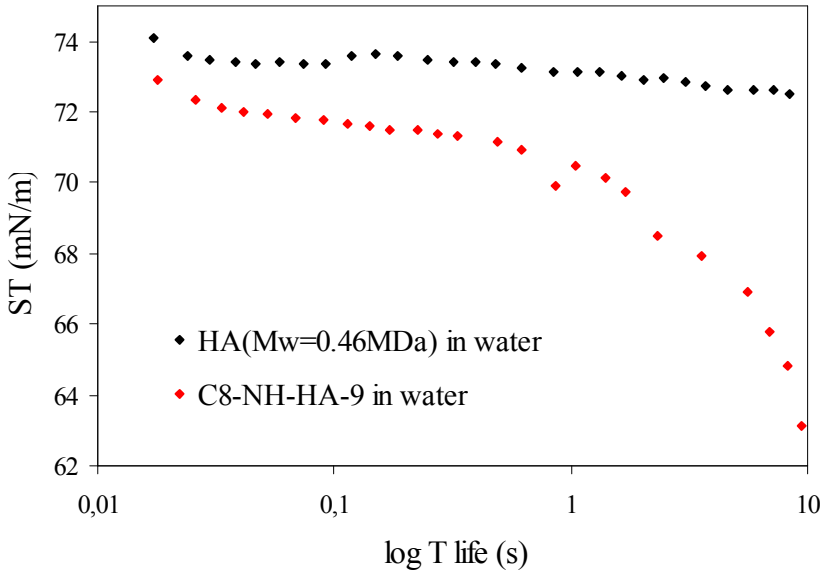

Figure 23 *ST vs. T life of hyaluronic acid and derivative C8-NH-HA-9 in water*

Derivatives C10-NH-HA-30 and C10-NH-HA-50 shows same surface tension dynamics movement like C8-NH-HA-9 in 0.15 M NaBr.

Each surfactant has different dynamic characteristics. As said before, the main role play different adsorption and diffusion velocity of various molecules on the bubble surface.

4.7 Diffusion constant

Hyaluronic acid and its derivatives have in low concentration relatively the same running of diffusion constant during the bubble life time. In the figure (Figure 24) is an example of hyaluronic acid in water, c = 0.1 g/l.

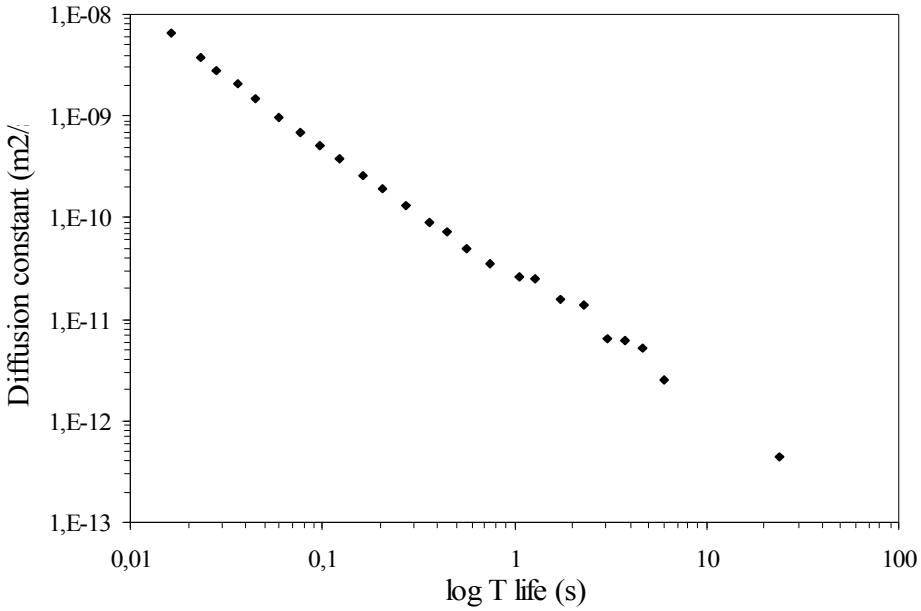

Figure 24 *Diffusion constant vs. log T life of hyaluronic acid in water, c = 0.1 g/l*

Higher concentrated solutions of hyaluronic acid and its derivatives show some fall during the measurement of all more concentrated samples. In the figure (Figure 25) is for example C8-NH-HA-9 in 0.15 M NaBr, c = 1 g/l. With increasing concentration of surfactants increase diffusion constant.

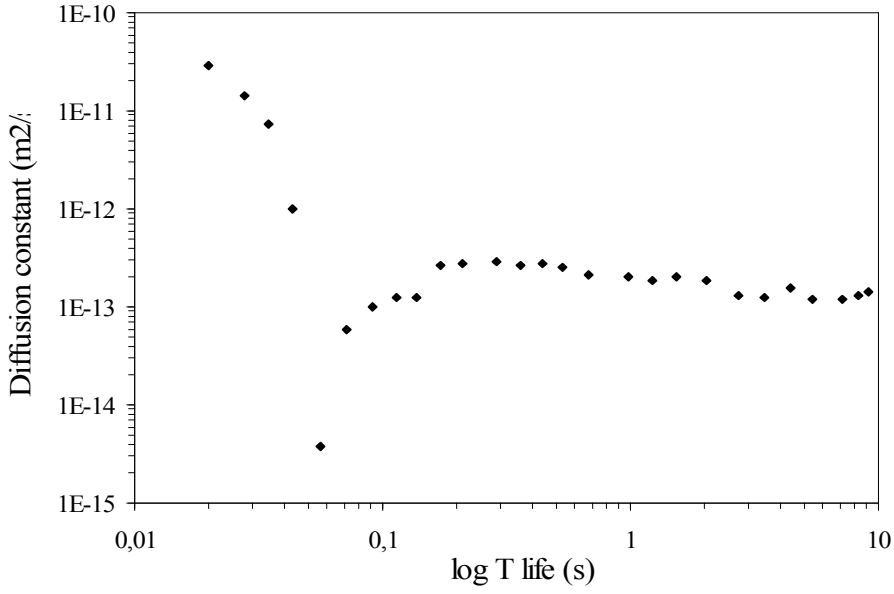

Figure 25 *Diffusion constant of C8-NH-HA-9 in 0.15 M NaBr, c = 1 g/l.*

5 CONCLUSION

Diploma thesis investigated biocolloids behaviour by the help of dynamic tensiometry method behind purpose measuring and analysis of conducd liquids surface from standpoint physical feature. Under examination was these biocolloids: HA (Mw = 0.4645 MDa; Mw = 1.69 MDa), derivatives (C8-NH-HA-9, C10-NH-HA-30 and C10-NH-HA-50). Records of this research can be draven up subsequently:

1. Methodology of maximum bubble pressure tensiometry for assesment liquids surface behaviour feature showed like suitable because measured solutions showed supposed behaviour of surface tension decrease in relation to liquid concentration.
2. For attestation of this method were proposed experimental measuring methods of above mentioned biocolloids. They were defined concentration series and suitable enviroment for surfactants.
3. There were measured concentration series of hyaluronic acid with different molecular mass (Mw = 0.4645 MDa; Mw = 1.69 MDa) and three derivatives of this acid (C8-NH-HA-9, C10-NH-HA-30 and C10-NH-HA-50). Further was these derivatives examined in five anions (Cl^-, I^-, Br^-, ClO_4^-, SO_4^{2-}) with various ionic strenght.
4. Experiments were measured with three methods. Standard method, increase flow rate method (IFR) and constant T life method (CT). For exclusion of errors was every sample measured with standard method and also with IFR method. Overall were made 372 experiments and about 14100 values were obtained. Each measurement took about 30 minutes. Experiments were assessed in 351 graps, which were transformed to the final summary graps.
5. Evaluation of all experiments was performed depending on dynamic behaviour of hyaluronans in colloid solutions. Sodium dodecyl sulphate was measured like model solution to find a new apparatus measurability. Results confirmed proposal behaviour of SDS depending on concentrations according to the article [17]. Correct apparatus functionality was proved by there measurements.

   It was found out that clean hyaluron acid is not surface active in measured concentrations however her derivatives (C8-NH-HA-9, C10-NH-HA-30 and C10-NH-HA-50) are. It depends on derivatives conentration and on solvent which was used. Further was found out that sodium hyaluronate in selected concentrations is not surface active too. Sodium hyaluronate surface activity was recorded as far as from concentration values more than 3 g/l, according to the article [18]. Derivate C8-NH-HA-9 was measured with anions (Cl^-, I^-, Br^-, ClO_4^-, SO_4^{2-}ions) with their concentration 0.15 M. In anion Cl^- was used another two concentrations (0.01 M a 0.5 M). It was discovered, that the I^- and the ClO_4^- ions do not influence the surface activity in depending on concentration however the Cl^-, Br^-, SO_4^{2-} ions do.

   In the end were measured next two derivatives of hyaluronic acid C10-NH-HA-30 and C10-NH-HA-50 where was recovered almost identical decrease of surface activity depending on increased concentration.

Surface tension dynamics was also studied. It was discovered that dynamics of low concentrated surfactants is slow, without any dramatic steps, compared to higher concentrated surfactants with complicated dynamics running. Great dynamic differences are for derivatives and SDS observed.

Finally the diffusion constant was calculated with increasing concentration of surfactants this constant increase too.

Results of this work are possible to use as starting results at experimental works with cross fade to the medical science research and pharmaceutical branch. I would recommend for next research to examine hyaluronic acid and her derivatives also in other concentrations, especially higher. Results of this work indicate that surface tension running of these surfactants will be fundamentals changing.

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7 LIST OF ABBREVIATIONS

HA	hyaluronic acid
SDS	sodium dodecyl sulphate
CMC	critical micelle concentration
NaCl	sodium chloride
NaBr	sodium bromide
NaI	sodium iodide
$NaClO_4$	sodium perchlorate
Na_2SO_4	sodium sulphate
H_2O	water
ST	surface tension
Mw	molecular weight
NaHA	sodium hyaluronate
HM	hydrophobically modified
SS	stock solution